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PATENT TRADEMARK OFFICE

Attorney's Docket No.: JYG149USA

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TRANSMITTAL LETTER TO THE U.S. ELECTED OFFICE  
(EO/US) - ENTRY INTO NATIONAL STAGE UNDER 35 USC 371

PCT/EP00/02887

28 March 2000

29 March 1999

International Application No.

International Filing Date

Priority Date Claimed

MICROSCALE TOTAL ANALYSIS SYSTEM

Title of Invention

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Applicant(s) for EO/US

## Box PCT

Assistant Commissioner for Patents  
Washington, DC 20231  
Attn: EO/US

Sir:

Applicant herewith submits to the United States Elected Office  
(EO/US) the following items under 35 USC 371:

- (1) This express request to immediately begin national examination procedures (35 USC 371(f)).
- (2) A copy of the cover sheet for the published International Application along with a copy of the specification as filed: 47 pages, including 8 pages of claims, 12 sheets of drawings, and a copy of the 3 page International Search Report.
- (3) a copy of the 4 page Request form.
- (4) a first Preliminary Amendment for entry prior to calculation of the filing fees.

Express Mail No. ET033435583US

- (5) our check in the amount of \$1004.00, covering the basic national fee as set forth in 37 CFR 1.492(a)(5) and based on the first Preliminary Amendment (28 total claims; 1 independent; and no multiple dependent).
- (6) A Second Preliminary Amendment.
- (7) Our check in the amount of \$666.00, covering the extra claim fees after entry of the second Preliminary Amendment (65 total claims; 3 independent; and no multiple dependent).

Copies of the following miscellaneous items are also enclosed:

- (8) Copy of the 4 page Demand for International Preliminary Examination.
- (9) Copy of the 5 page Written Opinion.
- (10) Copy of the 5 page Response to the Written Opinion.
- (11) Copy of the 7 page International Preliminary Examination Report.

The executed Combined Declaration and Power of Attorney form will be filed by the appropriate deadline under 37 CFR §1.495(c)(2) with the surcharge under 37 CFR §1.492(e).

Please charge any additional fees which may be required to effect entry into the National Phase and credit any overpayment to Deposit Account No. 08-3040.

Please direct all communications concerning this application to the undersigned.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:	)	
	)	Examiner:
Rossier et al.	)	
	)	Group Art Unit:
Application No.:	)	
	)	
Corresponding International Filing No.:	)	
PCT/EP00/02887	)	
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Filed: Herewith	)	
	)	
For: MICROSCALE TOTAL	)	
ANALYSIS SYSTEM	)	September 28, 2001

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Assistant Commissioner for Patents  
Washington, DC 20231

FIRST PRELIMINARY AMENDMENT

Sir:

Before calculating the filing fee, please amend the above-identified patent application as follows.

### In the Claims

Cancel claims 3-8, 11-14, 17, 18, 20, 21, 24, 30, 32, 34, 35, 37, 39, 40, 43, 45, 47, 50, 53, 55-57 and 59.

## REMARKS

Please enter this preliminary amendment before calculating the filing fee. This preliminary amendment cancels all the multiple dependent claims. Thus, after entry of this

Express Mail No. ET033435583US

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

preliminary amendment, the application has 28 claims in total, one independent claim, and no multiple dependent claims.

Charge any additional fees due to our deposit account no. 08-3040.

Respectfully submitted,  
Howson and Howson  
Attorneys for Applicant

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09/937690 00270  
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 JYG149USA

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:	)	
	)	Examiner:
Rossier et al.	)	
	)	Group Art Unit:
Application No.:	)	
	)	
Corresponding International Filing No.:	)	
PCT/EP00/02887	)	
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Filed: Herewith	)	
	)	
For: MICROSCALE TOTAL	)	
ANALYSIS SYSTEM	)	September 28, 2001

Box PCT  
 Assistant Commissioner for Patents  
 Washington, DC 20231

SECOND PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified patent application as follows.

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The format of this Amendment complies with 37 CFR §1.121 "Manner of making amendments in applications" as amended on November 7, 2000 pursuant to the "Patent Business Goals Final Rule". Thus, according to 37 CFR §1.121(c)(i), amended claims are provided in a form "without markings"; and according to 37 CFR §1.121(c)(ii), the amended claims are also provided, on a separate page, "marked up" to show the changes.

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In the Abstract

Please enter the abstract on a separate page as attached.

Express Mail No. ET033435583US

09/937690-030703

In the Claims

Amend claims 1, 9, 10, 15, 16, 22, 25, 26, 31, 33, 36, 38, 41, 44, 46, 48, 49, 51, 52, 54 and 58, as follows. [Format corresponding to 37 CFR §1.121(c)(i), ie. “**without markings**”.]

1(Amended). Apparatus for performing chemical assays involving aqueous fluids, the apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel into the reaction chamber, until such fluid is acted upon by a fluid entry force; wherein the gate means comprises at least a portion of the fluid inflow channel having a hydrophobic inner surface.

9(Amended). Apparatus according to claim 66, wherein each reaction chamber is provided with a separate inflow channel.

10(Amended). Apparatus according to claim 66, wherein there is one inflow channel forming a common conduit to all reaction chambers.

15(Amended). Apparatus according to claim 71, comprising a plurality of said apparatuses mounted together on a tape.

16(Amended). Apparatus according to claim 67, further comprising a substantially circular substrate, wherein the microchannels being arranged substantially radially, each microchannel having its inflow channel towards the circumference of the substrate and its opposite end communicating with a central chamber connected to said aspiration means.

22(Amended). Apparatus according to claim 77, wherein the reservoir communicates with the reaction chamber via a normally closed valve, which may be caused to open by the application of increased pressure to the aqueous fluid within the cavity.

25(Amended). Apparatus according to claim 78, wherein said conductive portion is formed of a conductive polymer material.

26(Amended). Apparatus according to claim 78, wherein said conductive portion is formed by an electrode.

31(Amended). Apparatus according to claim 79, wherein the detection means comprises at least one photodiode arranged along at least a portion of the reaction chamber.

33(Amended). Apparatus according to claim 82, wherein the reagent is selected from the group consisting of an oligonucleotide, a polypeptide, a protein, a natural molecule, and a synthetic molecule.

36(Amended). Apparatus according to claim 84, wherein the covalent bonding is achieved via a succinimide bonding agent.

38(Amended). Apparatus according to claim 85, wherein the crosslinker is polylysine.

41(Amended). Apparatus according to claim 87, wherein the substrate and overlying layer are formed of polymer materials, the melting point of at least one of the materials being

sufficiently low to permit the substrate and overlying layer to be sealed together by thermal lamination.

44(Amended). Apparatus according to claim 88, wherein the elastomeric material is polydimethylsiloxane (PDMS).

46(Amended). Apparatus according to claim 89, wherein the substantially opaque material comprises one of a carbon-filled polymer and a ceramics material.

48(Amended). A method according to claim 90, wherein the apparatus is formed from polymeric material.

49(Amended). A method according to claim 48, wherein the apparatus is formed by a technique selected from the group consisting of injection moulding, hot embossing, photoablation, casting, and polymerisation on a mould.

51(Amended). A method according to claim 91, wherein the overlying layer is sealed with the substrate by thermal lamination.

52(Amended). A method according to claim 90, wherein at least a part of the apparatus is formed of a material selected from the group consisting of ceramics material, glass, a conductor and a semi-conductor material.

54(Amended). A method according to claim 92, wherein the sample is caused to exit the reaction chamber before at least one of the reaction chamber and the expelled sample is monitored for at least one of the presence and concentration of a target substance.

58(Amended). A method according to claim 95, wherein the sample is expelled from the reaction chamber by spinning the substrate at a greater angular velocity, in the range 10 to 100,000 revolutions per minute, for a period of time in the range 1 to 100s.

Add new claims 60-96, as follows.

60(New). Apparatus according to claim 1, wherein the fluid inflow channel is formed in a substrate at least a portion of which is composed of a hydrophobic material.

61(New). Apparatus according to claim 1, wherein the fluid inflow channel is formed in a substrate at least a portion of which is physically treated to render it hydrophobic.

62(New). Apparatus according to claim 1, wherein the fluid inflow channel is formed in a substrate at least a portion of which is chemically treated to render it hydrophobic.

63(New). Apparatus according to claim 1, wherein the fluid inflow channel has a cross-sectional area in the range  $10\mu\text{m}^2$  to  $1,000\text{ mm}^2$ .

64(New). Apparatus according to claim 1, wherein the fluid inflow channel is moulded to be complementary in shape to a standard pipette.

65(New). Apparatus according to claim 1, wherein the fluid entry force is provided by piston pressure.

66(New). Apparatus according to claim 1, comprising a plurality of separate reaction chambers, each communicating with an inflow channel and associated gate means.

67(New). Apparatus according to claim 66, wherein each reaction chamber comprises a microchannel having at least one dimension in the range 1 to 1,000 $\mu$ m, and wherein each microchannel communicates, at its end distal the inflow channel, with a common conduit, the common conduit being connected to aspiration means adapted selectively to apply reduced pressure to the conduit and thus to draw fluid through the microchannels in operation.

68(New). Apparatus according to claim 10, wherein the common conduit has a cross-sectional area in the range 0.01 mm<sup>2</sup> to 25 cm<sup>2</sup>.

69(New). Apparatus according to claim 67, wherein the common conduit has a cross-sectional area in the range 0.01mm<sup>2</sup> to 25cm<sup>2</sup>.

70(New). Apparatus according to claim 67, wherein the microchannels are arranged generally parallel to each other.

71(New). Apparatus according to claim 70, wherein the microchannels are arranged generally perpendicularly to the common conduit.

72(New). Apparatus according to claim 66, wherein each reaction chamber comprises a microchannel having at least one dimension in the range 1 to 1,000 $\mu$ m, and further comprising a substantially circular substrate, wherein the microchannels are arranged substantially radially, the inflow channel being disposed toward the center of the substrate, and each microchannel having a waste chamber its opposite end toward the circumference of the substrate.

73(New). Apparatus according to claim 16, wherein the thickness of the substantially circular substrate is in the range 50 to 5,000 $\mu$ m.

74(New). Apparatus according to claim 72, wherein the thickness of the substantially circular substrate is in the range 50 to 5,000 $\mu$ m.

75(New). Apparatus according to claim 70, comprising a plurality of said apparatuses disposed on a rotatable support member, and wherein the fluid entry force is provided by centrifugal pressure as the support member is caused to rotate.

76(New). Apparatus according to claim 71, comprising a plurality of said apparatuses disposed on a rotatable support member, and wherein the fluid entry force is provided by centrifugal pressure as the support member is caused to rotate.

77(New). Apparatus according to claim 1, wherein the reaction chamber is provided, proximate thereto, with a sealed cavity forming a reservoir filled with an aqueous fluid.

78(New). Apparatus according to claim 1, wherein at least a portion of the surface of the reaction chamber is formed of an electrically conductive material, and the apparatus further comprises electrical detection circuitry connected to said conductive portion, to enable detection of a target species within the reaction chamber by electrochemical means.

79(New). Apparatus according to claim 1, further comprising electromagnetic radiation detection means adapted to detect radiation emitted by a target species in the reaction chamber.

80(New). Apparatus according to claim 79, wherein the detection means comprises at least one photomultiplier array arranged along at least a portion of the reaction chamber.

81(New). Apparatus according to claim 1, wherein a chemical reagent is immobilised on at least a portion of the inner surface of the reaction chamber, the reagent being adapted to interact with a target species whose presence is to be determined.

82(New). Apparatus according to claim 1, wherein a chemical reagent is immobilised on at least a portion of the inner surface of the reaction chamber, the reagent being adapted to interact with a target species whose concentration is to be determined.

83(New). Apparatus according to claim 82, wherein the reagent is adsorbed onto said inner surface of the reaction chamber.

84(New). Apparatus according to claim 82, wherein the reagent is covalently attached to said inner surface of the reaction chamber.

85(New). Apparatus according to claim 82, wherein the reagent is electrostatically attached to said inner surface of the reaction chamber via a crosslinker.

86(New). Apparatus according to claim 1, wherein at least a portion of one of the inner surface of the reaction chamber and the fluid inflow channel is provided with chemically function groups formed by one of a chemical and a physical treatment of the surface.

87(New). Apparatus according to claim 1, further comprising a substrate, in which at least one of the reaction chamber and the fluid inflow channel is formed as a depression in the substrate, and wherein the depression being sealed by an overlying layer applied over the substrate.

88(New). Apparatus according to claim 87, wherein the overlying layer is formed of an elastomeric material.

89(New). Apparatus according to claim 87, further comprising electromagnetic radiation detection means adapted to detect radiation emitted by a target species in the reaction chamber, and wherein at least a portion of the substrate is formed of a substantially opaque material and the overlying layer is formed of a substantially transparent material.

90(New). A method of manufacturing an apparatus for performing chemical assays involving aqueous fluids, comprising the steps of: forming at least one reaction chamber; and forming at least one fluid inflow channel communicating with the reaction chamber, at least a portion of the fluid inflow channel having a hydrophobic inner surface adapted to act as gate means to prevent passage of fluid through the fluid inflow channel into the reaction chamber until such fluid is acted upon by a fluid entry force.

91(New). A method according to claim 48, further comprising the steps of forming a substrate having at least one depression therein, and applying an overlying layer over the substrate to seal the depression so as to form one of a fluid inflow channel and a reaction chamber.

92(New). A method of operating an apparatus for performing chemical assays involving aqueous fluids, comprising the steps of:

utilizing an apparatus having at least one reaction chamber, at least one fluid inflow channel communicating with the reaction chamber, and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel into the reaction chamber until such fluid is acted upon by a fluid entry force, the gate means comprising at least a portion of the fluid inflow channel and having a hydrophobic inner surface;

placing at least one sample of an aqueous solution under test at the end of the fluid inflow channel distal the reaction chamber, at least a portion of the fluid flow channel having said hydrophobic inner surface;

causing the sample to enter the reaction chamber via the fluid inflow channel  
by applying a fluid entry force; and  
monitoring the sample in the reaction chamber for one of the presence and  
concentration of a target substance.

93(New). A method according to claim 92, wherein the sample is applied by one of a  
pipette, a syringe, and an electrically operated injector.

94(New). A method according claim 92, wherein the apparatus has a plurality of  
separate reaction chambers each communicating with an inflow channel and associated gate  
means, wherein each reaction chamber comprises a microchannel which communicates at its  
end distal the inflow channel with a common conduit, wherein the common conduit is  
connected to aspiration means, and wherein the fluid entry force is provided by said aspiration  
means, the aspiration means being activated to apply reduced pressure to each reaction  
chamber for a period of time in the range 0.1 to 100 seconds.

95(New). A method according to claim 94, wherein the apparatus has a substantially  
circular substrate, the microchannels are arranged substantially radially with respect to the  
substrate, and each microchannel having its inflow channel towards the circumference of the  
substrate and its opposite end communicating with a central chamber connected to said  
aspiration means; wherein the circular substrate is rotatable; and wherein the fluid entry force  
is provided by spinning the substrate at an angular velocity in the range 1 to 1,000 revolutions  
per minute for a period of time in the range 1 to 100 seconds.

96(New). A method according to claim 92, wherein the fluid entry force is provided by piston pressure.

[illegible]

**Version of Amended Claims  
with Markings to Show Changes Made  
Corresponding to 37 CFR §1.121(c)(ii)**

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1(Amended). Apparatus for performing chemical assays involving aqueous fluids, the apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the [or each] reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel[(s)] into the reaction chamber[(s)], until such fluid is acted upon by a fluid entry force; wherein the gate means comprises at least a portion of the [or each] fluid inflow channel having a hydrophobic inner surface.

9(Amended). Apparatus according to claim [8] 66, wherein each reaction chamber is provided with a separate inflow channel.

10(Amended). Apparatus according to claim [8] 66, wherein there is one inflow channel forming a common conduit to all reaction chambers.

15(Amended). Apparatus [comprising a plurality of apparatuses] according to claim [14] 71, comprising a plurality of said apparatuses mounted together on a tape.

16(Amended). Apparatus according to claim [11] 67, further comprising a substantially circular substrate, wherein the microchannels being arranged substantially radially, each microchannel having its inflow channel towards the circumference of the [circle] substrate and its opposite end communicating with a central chamber connected to said aspiration means.

22(Amended). Apparatus according to claim [21] 77, wherein the reservoir communicates with the reaction chamber via a normally closed valve, which may be caused to open by the application of increased pressure to the aqueous fluid within the cavity.

25(Amended). Apparatus according to claim [24] 78, wherein said conductive portion is formed of a conductive polymer material.

26(Amended). Apparatus according to claim [24] 78, wherein said conductive portion is formed by an electrode.

31(Amended). Apparatus according to claim [30] 79, wherein the detection means comprises at least one photodiode [or at least one photomultiplier array] arranged along at least a portion of the reaction chamber.

33(Amended). Apparatus according to claim [32] 82, wherein the reagent [comprises] is selected from the group consisting of an oligonucleotide, a polypeptide, a protein [or another], a natural [or] molecule, and a synthetic molecule.

36(Amended). Apparatus according to claim [35] 84, wherein the covalent bonding is achieved via a succinimide bonding agent.

38(Amended). Apparatus according to claim [37] 85, wherein the crosslinker is polylysine.

41(Amended). Apparatus according to claim [40] 87, wherein the substrate and overlying layer are formed of polymer materials, the melting point of at least one of the materials being sufficiently low to permit the substrate and overlying layer to be sealed together by thermal lamination.

44(Amended). Apparatus according to claim [43] 88, wherein the elastomeric material is polydimethylsiloxane (PDMS).

46(Amended). Apparatus according to claim [45] 89, wherein the substantially opaque material comprises one of a carbon-filled polymer [or] and a ceramics material.

48(Amended). A method according to claim [47] 90, wherein the apparatus is formed from polymeric material.

49(Amended). A method according to claim 48, wherein the apparatus is formed by a technique selected from the group consisting of injection moulding, hot embossing, photoablation, casting, [or] and polymerisation on a mould.

51(Amended). A method according to claim [50] 91, wherein the overlying layer is sealed with the substrate by thermal lamination.

52(Amended). A method according to claim [47] 90, wherein at least a part of the apparatus is formed of a material selected from the group consisting of ceramics material, glass, a conductor [or] and a semi-conductor material.

54(Amended). A method according to claim [53] 92, wherein the sample[(s)] is caused to exit the reaction chamber[(s)] before at least one of the reaction chamber[(s) or] and the expelled sample is monitored for at least one of the presence [or] and concentration of a target substance.

58(Amended). A method according to claim [57] 95, wherein the sample is expelled from the reaction chamber by spinning the substrate at a greater angular velocity, in the range 10 to 100,000 revolutions per minute, for a period of time in the range 1 to 100s.

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## REMARKS

After entry of the first and secondary preliminary amendments, the pending claims are claims 1, 2, 9, 10, 15, 16, 19, 22, 23, 25-29, 31, 33, 36, 38, 41, 42, 44, 46, 48, 49, 51, 52, 54, 58 and 60-96. This includes sixty-five claims in total, three independent claims, and no multiple dependent claims.

The Independent claims are claims 1, 90 and 92. Claim 1 is directed to an apparatus for performing chemical assays involving aqueous fluids. Claim 90 is directed to a method of manufacturing such an apparatus, and claim 92 is directed to a method of operating such an apparatus.


The claim amendments place the claims in better conformance with U.S. practice. No new subject matter was added. All the pending claims are supported by the disclosure provided by claims 1-59, as filed.

The attached abstract is substantially identical to the abstract published on the cover page of the published International application. No new matter was added.

Applicant respectfully requests consideration of claims 1, 2, 9, 10, 15, 16, 19, 22, 23, 25-29, 31, 33, 36, 38, 41, 42, 44, 46, 48, 49, 51, 52, 54, 58 and 60-96.

Charge any additional fees due to our deposit account no. 08-3040.

Respectfully submitted,  
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# ABSTRACT OF THE INVENTION

An apparatus for performing chemical assays in an aqueous medium. The apparatus contains a reaction chamber and a liquid in-flow channel connected to the chamber. The flow of liquid through the channel to the reaction chamber is controlled by the presence of a hydrophobic inner surface of the wall of the channel. Under normal conditions, fluid will not flow through the channel. However, application of an external force pushes the liquid through the channel into the reaction chamber.

The invention is applicable to the monitoring of many different molecular interactions, in particular molecular recognition between an immobilized affinity partner and a species in solution, such as immunoglobulin/antigen interaction, DNA hybridization, haptamer-protein interaction, drug and virus detection, high throughput screening of synthetic molecules and for determining the concentration and reaction kinetics of target species.

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This invention relates to apparatus for detecting the presence of a target species in an aqueous sample, and also to apparatus for determining the concentration and reaction kinetics of target species. The invention is applicable to the monitoring of many different molecular interactions, in particular molecular recognition between an immobilised affinity partner and a species in solution, such as immunoglobulin/antigen interaction, DNA hybridisation, haptamer-protein interaction, drug and virus detection and high throughput screening of synthetic molecules.

As many affinity complexations between two reaction partners are diffusion controlled, the time needed to reach reaction equilibrium is directly dependent on the mass transport of the molecule. The diffusion time of a molecule in a solution is proportional to the square of the path length; typically a small molecule needs less than one second to diffuse through 10  $\mu\text{m}$  while it needs two hours to traverse one millimetre. In order to decrease the equilibrium time of the reaction, the chemical partners must therefore be placed as close as possible to each other; by reducing the reactor size to microdimensions, immobilising one partner on the surface of the reactor and filling the reactor with the second partner, the equilibrium time can be dramatically decreased.

The use of microreactors not only enhances the speed of affinity assays, but also facilitates the obtaining of information concerning reaction kinetics, which is important in the understanding of the thermodynamic stability of complexes. The affinity constant  $K_a$  is the ratio between the forward and reverse reaction rate constants  $k_1$  and  $k_{-1}$ , which represent the association and the dissociation constants respectively. A strong complexation is characterised by a very fast association and a very slow dissociation, which in the particular case of sorbent affinity assays are adsorption and desorption from the

- 2 -

surface of the microreactor. The understanding of these thermodynamic properties can be used for the study of cross-reactivity between several antigens or of non-specific adsorption of a matrix element during an affinity assay. By modulating the incubation time of the solution, the complexation of higher affinity partners is favoured and the non-specific adsorption is then reduced to a minimum. This fact may be an important factor in the decrease of the detection limit in immunosorbent assays due to the minimisation of the background signal. This method can also be applied to monitor the adsorption of antigens of different molecular weight.

As the diffusion coefficient of a molecule is proportional to its mass, the diffusion time of the molecule through the reaction chamber is different for small and large molecules. In the case of molecules of different molecular weight with the same epitope, (e.g. fibrine degradation products), the  $K_d$  may be the same for all molecules, whereas the diffusion coefficient is different for each of them. When a kinetics experiment is carried out, the smaller molecules will quantitatively reach the antibodies before the larger ones. The monitoring of the signal resulting from the affinity reaction as a function of time can thus deliver useful kinetics information, which can help in understanding the degradation process. These kinetics events can be followed by modulating the residence time of molecules in contact with their reaction partners, which can most readily be achieved by immobilising an antibody on the walls of a series of microreactors and by incubating different solutions of the analyte of interest for different periods of time.

In the past, analytical procedures of the type described above (such as enzyme-linked immunosorbent assays - ELISAs) have been performed using microtiter plates and have been relatively slow. In recent years, great efforts have been made to reduce the size of analytical devices to micrometer scale with the effect of reducing reaction times.

These miniaturised systems have been termed "microscale total analysis systems" ( $\mu$ -TAS)<sup>1</sup>, and they have already been recognised as convenient means of manipulating and analysing small sample quantities<sup>2-8</sup>. Most  $\mu$ -TAS devices to date  
5 have been produced by photolithography, wet chemical etching or thin film deposition on substrates such as glass, quartz and silicon<sup>9, 10</sup>. In order to decrease production costs, plastic substrates have also been micromachined using either silicone rubber casting<sup>11-14</sup> injection moulding<sup>15</sup> embossing  
10<sup>16, 17</sup> or laser photoablation<sup>18</sup>. These structures are planar devices with channels of micrometre size that are often sealed by thermal or anodic bonding to a glass cover. Interconnected channels may be fabricated easily, which makes possible the rapid separation and reactions in volumes  
15 of few a picoliters. Other advantages of  $\mu$ -TAS are the reduction of sample and reagent consumption and the increase of precision and reproducibility relative to bench scale apparatus<sup>21, 22</sup>.

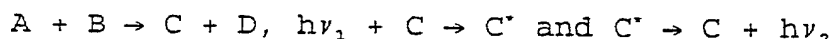
Competitive immunoassays have also been performed on  
20 micro-chips<sup>23-25</sup>, but the micro-channels have only been used to electrophoretically separate free and bound forms of antigen or antibody. In these assays, antibody and labelled antigen are added in specific quantities to the sample to be analysed. The sample is then incubated with a  
25 mixture of the labelled and native antigens that compete for a limited number of antibody binding sites. The micro-channel is then used to separate the free labelled antigen from the complex by capillary electrophoresis, and quantitation is performed by luminescence (fluorescence or  
30 chemiluminescence) at the end of the separation channel. The amount of free labelled antigen measured is then related to the analyte concentration in the sample using a previously determined calibration curve. In this type of assay it is essential to avoid adsorption of a reaction partner on the  
35 micro-channel walls.

Another type of immunoassay device has been developed for simultaneous analysis of multiple samples<sup>26</sup>. In this

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case, biotin-labelled antibodies are patterned onto an  
avidin-coated waveguide so as to form an array of six  
vertically oriented stripes of captured antibodies  
immobilised on the waveguide surface by avidin-biotin  
5 bridges. Samples are then analysed using a sandwich  
immunoassay format by patterning another array of six  
horizontally oriented lines containing the corresponding  
fluorescent-labelled antigen at various concentrations.  
Fluorescent complexes on the surface of the waveguide are  
10 then excited by a diode laser, and the fluorescence  
intensities of the 36 square dots is collected by a CCD  
camera. This immunosensor allows the analysis of multiple  
samples in parallel and simultaneous detection of more than  
one analyte per sample.

15 Numerous analytical methods utilise luminescence to  
detect an analyte of interest. Luminescence is the generic  
term referring to the emission of an electromagnetic  
radiation (UV, visible or IR) by an excited molecule that  
relaxes to its ground state, can be induced by  
20 photoexcitation (photoluminescence) or by a chemical  
reaction (chemiluminescence and electrochemiluminescence).  
Chemifluorescence (CF) is another class of luminescent  
reactions which combines the reaction mechanisms of both PL  
and CL. In this case, a fluorogenic substrate A is converted  
25 to a fluorescent product C by chemical reaction, and  
luminescence is generated by excitation of this product:



For analytical purposes, one of the reactants of the  
assay system that is capable of generating luminescence can  
30 be attached to a molecule in order to "label" it  
specifically. The presence or absence of an observable label  
attached to one or more of the binding materials is then  
used as an indicator of the existence of an analyte of  
interest. A large body of experiments has been developed to  
35 detect and quantitate trace amounts of pharmaceuticals,  
microorganisms, hormones, viruses, antibodies, nucleic acids

and other proteins by such methods. In clinical diagnostics for instance, competitive and sandwich immunoassays using luminescence detection are now used on a routine basis <sup>27, 28</sup>.

In enzyme-mediated immunoassays, a molecule is labelled with an enzyme that catalyzes the luminescence reaction. Typical examples are the detection of immunoreagents labelled with Horse Radish Peroxidase (HRP) or Alkaline Phosphatase (ALP) which, in the presence of hydrogen peroxide and hydroxide ions, respectively facilitate the oxidation of luminol and dioxetanes and the hydrolysis of phosphate-containing reagents. Similarly, ALP has been used in CF assays to cleave a phosphate group from a fluorogenic substrate to yield a highly fluorescent product <sup>29</sup>.

Luminescence assay methods are widely used in the analysis of peptides, proteins, and nucleic acids. CL has been shown to be a highly sensitive detection method in both flow injection analysis (FIA) and high-performance liquid chromatography <sup>30-32</sup>, and it has also been employed in capillary electrophoresis (CE) <sup>33, 34</sup> for the detection of amino acids neurotransmitters <sup>35</sup>, rare-earth metal ions <sup>36</sup> or labelled proteins <sup>37</sup>. However, it is in immunoassays that luminescence is the most commonly used detection method <sup>27, 34, 38-43</sup>.

Among prior art methods for the measurement of enzymatic reaction rate, US 4,621,059 discloses a method in which the light emitted by a luminescent substance flowing through a capillary column and reacting with an immobilised enzyme is collected through a plurality of optical fibers that are arranged along the longitudinal direction of the column in order to determine the enzyme activity or the quantity of analyte of interest from the distribution of the luminescence intensity.

US 5,624,850 describes a method for performing immunoassays in capillaries in which fluorescence is used to detect an analyte of interest in translucent capillaries

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having an inner diameter from  $\sim 0.1 \mu\text{m}$  to  $1.0\text{mm}$ . Similarly, homogeneous chemiluminescence immunoassays can be carried out, for example as described in US 5,017,473, in which a light absorbing material and a luminescent labelled tracer are incubated with the analyte/anti-analyte complex, so that all the emitted light is absorbed by the light-absorbing material except that associated to the bound tracer.

A method is disclosed in US 5,585,069 in which two or more samples are processed in parallel in a system comprising a plurality of wells that are connected by one or more channels to move a sample from one well to the other using mechanical or electrokinetic pumping. In this apparatus, the channels are simply used as connections between two wells, and are not used as reaction or detection chambers.

One of the main difficulties associated with the use of  $\mu$ -TASS is the proper mixing of reagents, due to the low Reynolds numbers of the flows. Another difficulty resides in the accurate timing of fluid entry, which is essential for kinetics studies. In addressing these problems, the applicants have found that fluid entry into a reaction chamber (for example a microchannel) can be accurately be controlled by means of a hydrophobic gate.

The present application therefore provides, in one aspect, apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the or each reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel(s) into the reaction chamber(s), until such fluid is acted upon by a fluid entry force; wherein the gate means comprises at least a portion of the or each fluid inflow channel having a hydrophobic inner surface.

Preferably, the apparatus has a plurality of reaction chambers, which take the form of microchannels, each having an associated fluid inflow channel. Alternatively, a

plurality of microchannels may be served by a single inflow channel, feeding into a common conduit communicating with the microchannels. In some preferred embodiments, the fluid entry force is provided by aspiration means connected to a common conduit communicating with each microchannel at its end distal the inflow channel. In other embodiments, the apparatus comprises a rotatable support member and the fluid entry force is provided by centrifugal force upon rotation of the substrate. Conveniently, the support member may form the substrate of the microchannel apparatus, with the microchannels being arranged generally radially. Alternatively the rotatable support member may serve as a support for one or more devices having parallel microchannels.

15 The advantage of a common source of fluid entry force for all of the microchannels is that simultaneous filling may be ensured, the fluid samples being prevented from entering the microchannels by the hydrophobic gate means until the fluid entry force is applied. Furthermore, the degree of fluid entry force may also readily be controlled, to ensure rapid filling of the microchannels, and adequate mixing. The microchannels may also be emptied in an efficient and rapid manner, by application of an increased force to the fluid in the channels, for example by increasing the degree of aspiration, or by increasing the rate of rotation of the rotation support member. An exact end point of an assay may thereby be achieved. In many instances it is advantageous for the sample to be expelled before monitoring for bound target species.

30 If desired, a liquid reagent or a washing fluid may be supplied in a sealed cavity forming a reservoir, there preferably being one such reservoir per microchannel. The reservoirs may be arranged to communicate with their respective microchannels via normally closed valves, and may be caused to expel their contents through such valves when acted upon by respective pistons. Alternatively, there may

Detection of target species with the microchannels may be achieved by conventional means. For example, to permit 5 electrochemical detection, preferred embodiments of apparatus are constructed so that at least a portion of the surface of the microchannel is formed of an electrically conductive material. This may for example be a conductive polymer material or an electrode. In some embodiments, at 10 least a portion of the microchannel walls may be formed of a semi-conductor material such as indium oxide. Preferably, the semi-conductor material is transparent. Alternatively, detection may be achieved by luminescence or fluorescence means, in which case an electromagnetic radiation detector, 15 such as a photodiode or a photomultiplier, is provided.

The invention also extends to a method of manufacturing an apparatus as defined above, comprising the following steps which may be performed in either order or 35 simultaneously: forming at least one reaction chamber; and forming at least one fluid inflow channel communicating with

the reaction chamber(s), at least a portion of the or each fluid inflow channel having a hydrophobic inner surface adapted to act as gate means to prevent passage of fluid through the fluid inflow channel into the reaction chamber(s) until such fluid is acted upon by a fluid entry force.

For ease of fabrication, the apparatus is preferably formed in two main parts: a substrate in which the microchannels (and possibly also the inflow channels) are formed as depressions (for example by injection moulding, hot embossing, photoablation, casting or polymerisation on a mould); and an overlying layer applied over the substrate and over the depressions, to form the microchannels (and optionally also the inflow channels). In embodiments in which the inflow channels are not produced in the substrate they may, for example, be produced by drilling through a laminated overlying layer using a laser, or by depositing above the inlet of the reaction chamber a joint made of a hydrophobic material such as polydimethylsiloxane (PDMS).

The apparatus may be formed from any suitable material, for example, ceramics, glass, semiconductors, polymers, or combinations thereof. In a particularly preferred embodiment, both the substrate and lamination layer are formed of polymer material, which not only permits ready formation of the microchannels (for example by photoablation), but also allows the two components to be fused together by a thermal lamination technique. For this purpose, it is preferred that at least one of the polymers is of a material which has a relatively low melting point, for example polyethylene with a melting point of under 200°C. The lamination layer may with advantage be of an elastomeric material, such as polydimethyl siloxane (PDMS). In apparatus for use in conjunction with optical detection means, it is preferred that the lamination layer be formed of a substantially transparent material, and the substrate of a substantially opaque material (such as a ceramics material or a carbon-filled polymer).

10 In embodiments of apparatus having aspiration means, the fluid entry force is preferably applied by activating the aspiration means to apply reduced pressure to the microchannels for a period of time in the range 0.1 to 100s. In order to evacuate the microchannels, the aspiration means  
15 may then be activated to provide an even lower pressure to the microchannels, optionally in conjunction with the supply of washing fluid from a reservoir.

The invention is hereinafter described in more detail by way of example only, with reference to the accompanying drawings, in which:

30 Fig 1 is a schematic cross section of an embodiment of apparatus according to the invention illustrated a) after deposition of an aqueous sample drop on the hydrophobic gate, and b) after sample loading;

5            Fig 3 is a schematic plan view of an alternative embodiment of apparatus according to the invention, in which parallel filling and washing step are achieved by centrifugal force;

Fig 5a is a partial plan view of an embodiment of apparatus according to the invention, incorporating a fluid reservoir adjacent the fluid inflow channel;

Fig 6 is a top plan representation of an embodiment of apparatus according to the invention manufactured by UV-Laser photoablation of a polycarbonate compact disk, the embodiment being constructed substantially as the apparatus 25 of Fig 3;

30 Fig 8 is a graph illustrating fluorescence results indicating the level of binding between DDi-ALP in a test involving use of two microchannels of apparatus according to

Fig 9 is a fluorescence image obtained using apparatus of the type illustrated in Fig 6, in which different 5 microchannels were incubated with different concentrations of DDi-ALP;

Fig 11 is a graph illustrating variation of fluorescence intensity with time from an incubation of ALP-DDi in an embodiment of apparatus according to the invention, having at least one antibody coated microchannel;

The microchannel devices of Figs 1 to 6 are produced by UV-Laser photoablation of commercially available polymers such as PET or polycarbonate. The photoablation procedure is performed in known fashion, for example as described previously by the present applicants<sup>44</sup>. Briefly, a polymer sheet is rinsed with distilled water and ethanol and then mounted on an X,Y machining stage (Microcontrol, France). UV-Laser pulses (193 nm) (Lambda Physik LPX 205 i, Germany) are then fired at the polymer substrate target through a photomask and a 10:1 lense with a frequency of 50 Hz at 200 mJ/pulse, corresponding to a fluence per pulse of 1 J/cm<sup>2</sup> on the surface. During the photoablation process, the polymer substrate is moved horizontally with a X,Y stepping motor (Microcontrol, France) at a speed of 0.2 mm/s resulting in

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linear channels 22 mm long. The microchannels are typically between 1 and 1,000 $\mu$ m in width, and in this example are approximately 100 $\mu$ m wide. The depth of the channels was fixed at 40  $\mu$ m, by controlling the number of laser pulses 5 used (each pulse photoablates approximately 150 nm). The channels are then sealed by thermal lamination of a layer of polyethylene over the base polymer sheet, the channels then exhibiting a trapezoidal shape in which three walls are composed of the substrate polymer (PET or Polycarbonate) and 10 the top is composed of the lamination (Polyethylene). Fluid inflow channels (or "gates") (1) are opened either by firing enough laser pulses or are mechanically drilled through the hydrophobic lamination layer. The gates, which may have a diameter between 10 $\mu$ m and 10mm, have hydrophobic inner 15 surfaces due to the nature of the polymer, and therefore inhibit passage of aqueous fluids.

The precise arrangement of microchannels is not crucial to the operation of the invention, though two general geometries have been developed and tested by the applicants 20 and proved to be of benefit. In the first of these, a plurality of microchannels are arranged parallel to each other, conveniently on a generally rectangular substrate. The inflow channel "gates" of the various microchannels are aligned with each other, to permit rapid and efficient 25 loading with test solutions from a linear multiple pipette device (see Fig 2). In the second configuration, the microchannels are arranged radially on a generally circular substrate, either with the inflow channel gates towards the centre of the circle and the opposite (outflow) ends of the 30 microchannels towards the circumference (Figs 3 and 6), or vice versa (Fig 4).

A number of different means may be employed to provide the fluid entry force, of which the preferred means are aspiration and centrifugal force. In the apparatus of Fig 35 2, a common conduit (3) is supplied at the outflow ends of the microchannels (2), to which a reduced pressure is applied during operation of the device, to draw fluid into

Typically, in an aspiration driven device (as in Figs 2 and 4) a 2  $\mu$ l sample is placed with a pipette on each gate (1). The solution is then loaded into the microchannel by a brief aspiration from the common conduit (3;6). This technique ensures homogeneity of the solution over the whole microchannel. After incubation, the microchannel is aspirated and rinsed three times with 2  $\mu$ l. It is worth noting that the washing solution volume is much larger than that of the microchannel (about 100 nl) thus ensuring efficient washing. Using devices driven by centrifugal pressure, the filling and washing procedures may be achieved by placing 2  $\mu$ l of solution over each gate (1). Slow rotation results in loading of the sample into the microchannel(s), and faster rotation is subsequently used to expel the sample from the microchannel(s).

Fig 5 illustrates an optional modification of apparatus according to the invention, in which each microchannel has an associated fluid reservoir (10) formed by a sealed cavity situated adjacent the fluid inflow gate (1). The reservoir communicates with microchannels (2) by means of a normally closed valve (12), which comprises valve member (13) which may be deformed under pressure into depression (14). Reservoir (10) is capped by seal (15), which may be broken by downward pressure applied by piston (11), which is profiled to be a close fit within reservoir (10). Downward

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movement of piston (11) within reservoir (10) increases the fluid pressure within the reservoir, thus opening valve (12) and allowing fluid from the reservoir to enter the microchannel. Depending upon the requirements of any particular assay, the reservoir may either be filled with a reagent or with wash fluid.

By way of example, various tests were carried out to establish the utility of apparatus according to the invention in performing an immunoassay for D-Dimer. D-Dimer is used as a diagnostic indicator in thromboembolic events: deep vein thrombosis and pulmonary embolism can be diagnosed by monitoring D-Dimer concentration in blood. In the past, the most reliable assay of D-Dimer have been performed by ELISA techniques, for example the "Asserachrom D-Di" of Diagnostica Stago. However, standard ELISA techniques are not suited for emergency situations, and alternative membrane-based techniques have been developed which use colour based detection systems <sup>48</sup>. However, these suffer from the disadvantage that the detection mechanism is too subjective.

In the present tests, the detection of the enzyme was effected by a chemifluorescent substrate solution (VCR, Amersham). This system is based on the fluorescent detection of the AttoPhos substrate hydrolysed by ALP. The microchannels were then exposed to a Fluorescence Imager screen (MP840, Molecular Dynamics) and every channel was read for 1 minute. The image was then quantified using Image Quant software (Molecular Dynamics). The calibration of the enzyme in the microchannel was achieved by mixing the substrate solution with different concentrations of enzyme and incubating for 5 minutes. The microchannels were then filled with the mixtures and analysed with the fluorescence imager. In the actual tests, the enzyme was immobilised on the surface of the microchannels, and the VCR solution was added to the channels with fluorescence being measured 5 minutes later.

Immobilisation of the proteins was achieved by physisorption for 1 hour at room temperature. The mouse IgG antibody (Serbio, France) was immobilised by placing either 10 or 100  $\mu\text{g/ml}$  in the microchannel, followed by incubation 5 for 1 hour in a wet chamber. The surface was then washed with PBS and 20 % Tween (Tween/Water :0.2 ml/L, Fischer Germany), and blocked for 1 hour with a solution of 50  $\mu\text{g/ml}$  of heat shocked BSA (Sigma, USA) in the washing buffer solution. After another washing step, the channels were 10 individually filled with the antigen solution. After five minutes (except for the kinetic experiment, where other periods are specified below), the microchannels were rinsed and a solution of 10  $\mu\text{g/ml}$  of alkaline phosphatase labelled antigen (ALP-DDi) was introduced and rinsed again after five 15 minutes.

Fluorescence dependence on enzyme concentration after 5 minutes of incubation is presented graphically in Figure 7. The detection limit is reached in the range of 1  $\text{ng.ml}^{-1}$ . The non-linear detection range is due to the fact that the 20 product of the hydrolysis is not highly soluble and may precipitate on the surface at higher concentrations. Nevertheless, this system can be used for quantifying the enzyme concentration in the microchannel.

In order to study the activity of the adsorbed 25 antibodies in the microchannel, two different incubation procedures were undertaken. Firstly, a few channels were incubated only with BSA. Secondly, some further channels were incubated with Ab and then with BSA. Every channel was then filled with the DDi-ALP, incubated for 1 hour and 30 washed by aspiration following the procedure described above. The fluorescence intensity of each channel was then measured and the results are presented in Figure 8. The channels incubated only with BSA exhibited a low fluorescence which is not significantly different from that 35 of the polymer substrate itself. In contrast the channels incubated with the antibodies were much more fluorescent, thereby demonstrating that DDi-ALP was adsorbed on the

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antibodies. This experiment shows that some of the adsorbed antibodies are still active on the surface and that BSA is an effective blocker against the non-specific adsorption of the DDi-ALP complex.

5        Figure 9 shows the fluorescence of the substrate in the channels after adsorption of different concentrations of ALP-DDi on the  $10 \mu\text{g}.\text{ml}^{-1}$  adsorbed antibodies. The fluorescence intensity of the microchannel lines clearly shows the gradient of concentration in the different  
10 microchannels. The relative intensity of every microchannel is shown graphically in Figure 10. Saturation of channels is reached at about  $30 \mu\text{g}/\text{ml}$ .

Figure 11 shows the fluorescence intensity of microchannels that have been incubated for different periods  
15 of time. For short incubation times ( $<5 \text{ min}$ ), the intensity grows linearly, showing that the antigens are very quickly captured by the antibodies. It is thought that all the antigens have still not reached the surface by diffusion. This first slope approximately follows the diffusion of the  
20 molecules to the walls. The molecules then react rapidly and the reaction becomes quasi diffusion-controlled. After 5 minutes of incubation, the reaction is controlled by slower kinetics driven by two different phenomena. Firstly, large molecules diffuse much more slowly and therefore reach the  
25 surface after a long time. In this case, the molecules can be partially degraded fibrin products, of which the molecular weight can be larger than  $1000 \text{ kD}$ . Secondly, there is a tendency for non-specific adsorption; such reactions are much slower than immunological recognition and are  
30 driven by electrostatic or hydrophobic interactions that require reorganisation at the molecular level. This type of non specific adsorption may therefore be excluded by short incubation times.

Figure 12 shows the fluorescence dependence of the D-  
35 Dimer concentration after a competitive immunoassay. In the low concentration range, most of the immobilised antibody

10 49 .

These experiments demonstrate the feasibility of ELISA techniques in microchannels. Benefitting from fast equilibration times and rapid filling and rinsing procedures, the time taken to complete an assay (including 15 calibration) may be reduced to less than 10 minutes, compared to a typical time of 3 hours for an ELISA in a microtiter plate. Hundreds of microchannels may be provided on a substrate, if desired, and the ability to ensure simultaneous filling provides the possibility of highly 20 efficient parallel assays.

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1. Apparatus for performing chemical assays involving aqueous fluids, the apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the or each reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel(s) into the reaction chamber(s), until such fluid is acted upon by a fluid entry force; wherein the gate means comprises at least a portion of the or each fluid inflow channel having a hydrophobic inner surface.

15        3. Apparatus according to claim 1 or claim 2, wherein  
the fluid inflow channel is formed in a substrate at least  
a portion of which is composed of a hydrophobic material.

5. Apparatus according to any preceding claim, wherein the fluid inflow channel has a cross-sectional area in the range  $10\mu\text{m}^2$  to  $1,000\text{mm}^2$ .

7. Apparatus according to any preceding claim,  
30 wherein the fluid entry force is provided by piston  
pressure.

5        9. Apparatus according to claim 8, wherein each  
reaction chamber is provided with a separate inflow channel.

10 11. Apparatus according to claims 8, 9 or 10, as  
appendant to claim 2, wherein each microchannel  
communicates, at its end distal the inflow channel, with a  
common conduit, the common conduit being connected to  
aspiration means adapted selectively to apply reduced  
15 pressure to the conduit and thus to draw fluid through the  
microchannel in operation.

20        13. Apparatus according to any preceding claim,  
wherein the microchannels are arranged generally parallel to  
each other.

14. Apparatus according to claim 13, as appendant to any of claims 10 to 12, wherein the microchannels are arranged generally perpendicularly to the common conduit.

15. Apparatus comprising a plurality of apparatuses according to claim 14 mounted together on a tape.

16. Apparatus according to claim 11, comprising a substantially circular substrate the microchannels being  
30 arranged substantially radially, each having its inflow channel towards the circumference of the circle and its

opposite end communicating with a central chamber connected to said aspiration means.

17. Apparatus according to claim 8 or claim 9, comprising a substantially circular substrate the 5 microchannels being arranged substantially radially, the inflow channel(s) being disposed towards the centre of the circle, and each microchannel having a waste chamber at the opposite end towards the circumference of the circle.

18. Apparatus according to claim 16 or claim 17, 10 wherein the thickness of the substantially circular substrate is in the range 50 to 5,000 $\mu$ m.

19. Apparatus according to claim 16, wherein the circular substrate is rotatable, and the fluid entry force is provided by centrifugal pressure as the substrate is 15 caused to rotate.

20. Apparatus comprising a plurality of apparatuses according to claim 13 or claim 14 disposed on a rotatable support member, and wherein the fluid entry force is provided by centrifugal pressure as the support member is 20 caused to rotate.

21. Apparatus according to any preceding claim, wherein the or each reaction chamber is provided, proximate thereto, with a sealed cavity forming a reservoir filled with an aqueous fluid.

22. Apparatus according to claim 21, wherein the 25 reservoir communicates with the reaction chamber via a normally closed valve, which may be caused to open by the application of increased pressure to the aqueous fluid within the cavity.

23. Apparatus according to claim 22, further 30 comprising a piston member having an outer profile shaped to fit within the cavity, the cavity being capped by a

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5 reaction chamber.

10 circuitry connected to said conductive portion, to enable  
detection of a target species within the reaction chamber by  
electrochemical means.

15 material.

conductive portion is formed by an electrode.

electrode is of a semiconductor material.

20        28. Apparatus according to claim 27, wherein said  
semiconductor material is substantially transparent.

conductor material is indium oxide.

25 further comprising electromagnetic radiation detection means adapted to detect radiation emitted by a target species in the reaction chamber.

30 least one photomultiplier array arranged along at least a portion of the reaction chamber.

34. Apparatus according to claim 32 or claim 33,  
10 wherein the reagent is adsorbed onto said inner surface of  
the reaction chamber.

33. Apparatus according to claim 32, wherein the reagent comprises an oligonucleotide, a polypeptide, a protein or another natural or synthetic molecule.

34. Apparatus according to claim 32 or claim 33,  
10 wherein the reagent is adsorbed onto said inner surface of  
the reaction chamber.

35 Apparatus according to claim 32 or claim 33,  
wherein the reagent is covalently attached to said inner  
surface of the reaction chamber.

15        36. Apparatus according to claim 35, wherein the  
covalent bonding is achieved via a succinimide bonding  
agent.

37. Apparatus according to claim 32 or claim 33,  
wherein the reagent is electrostatically attached to said  
20 inner surface of the reaction chamber via a crosslinker.

38. Apparatus according to claim 37, wherein the crosslinker is polylysine.

39. Apparatus according to any preceding claim, wherein at least a portion of the inner surface of the  
25 reaction chamber and/or of the fluid inflow channel is provided with chemically function groups formed by chemical or physical treatment of the surface.

40. Apparatus according to any preceding claim, comprising a substrate, in which the reaction chamber and/or the fluid inflow channel are formed as a depression(s), the

41. Apparatus according to claim 40, wherein the substrate and overlying layer are formed of polymer materials, the melting point of at least one of the materials being sufficiently low to permit the substrate and overlying layer to be sealed together by thermal lamination.

10        43. Apparatus according to claim 40 or claim 41,  
      wherein the overlying layer is formed of an elastomeric  
      material.

15        45. Apparatus according to claim 40, as appendant to  
claim 30 or 31, wherein at least a portion of the substrate  
is formed of a substantially opaque material and the  
overlying layer is formed of a substantially transparent  
material.

47. A method of manufacturing an apparatus according to any of claims 1 to 46, comprising the following steps which may be performed in either order or simultaneously: forming at least one reaction chamber; and forming at least one fluid inflow channel communicating with the reaction chamber(s), at least a portion of the or each fluid inflow channel having a hydrophobic inner surface adapted to act as a gate means to prevent passage of fluid through the fluid inflow channel into the reaction chamber until such fluid is acted upon by a fluid entry force.

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48. A method according to claim 47, wherein the apparatus is formed from polymeric material.

49. A method according to claim 48, wherein the apparatus is formed by injection moulding, hot embossing, photoablation, casting, or polymerisation on a mould.

50. A method according to claim 48 or claim 49, comprising the steps of forming a substrate having at least one depression therein, and applying an overlying layer over the substrate to seal the or each depression so as to form at least one fluid inflow channel and/or at least one reaction chamber.

51. A method according to claim 50, wherein the overlying layer is sealed with the substrate by thermal lamination.

52. A method according to claim 47, wherein at least a part of the apparatus is formed of a ceramics material, glass, a conductor or a semi-conductor material.

53. A method of operating an apparatus according to any of claims 1 to 46, comprising the steps of: placing at least one sample of an aqueous solution under test at the end of at least one fluid inflow channel distal at least one reaction chamber; causing the sample to enter the reaction chamber(s) via the fluid inflow channel(s) by applying a fluid entry force; and monitoring the sample in the reaction chamber(s) for the presence or concentration of a target substance.

54. A method according to claim 53, wherein the sample(s) is caused to exit the reaction chamber(s) before the reaction chamber(s) or the expelled sample is monitored for the presence or concentration of a target substance.

\* , at least a portion of the or each fluid inflow channel having a hydrophobic inner surface

55. A method according to claims 53 or 54, wherein the  
or each sample is applied by means of a pipette, a syringe,  
or an electrically operated injector.

56. A method according to any of claims 53 to 55, for  
5 operating an apparatus according to claim 11 or any claim  
appendant thereto, wherein the fluid entry force is provided  
by aspiration means, the aspiration means being activated to  
apply reduced pressure to the or each reaction chamber for  
a period of time in the range 0.1 to 100s.

10 57. A method according to any of claims 53 to 55, for  
operating an apparatus according to either claim 19 or claim  
20, or any claim appendant thereto, wherein the fluid entry  
force is provided by spinning the substrate or the support  
member at an angular velocity in the range 1 to 1,000  
15 revolutions per minute for a period of time in the range 1  
to 100s.

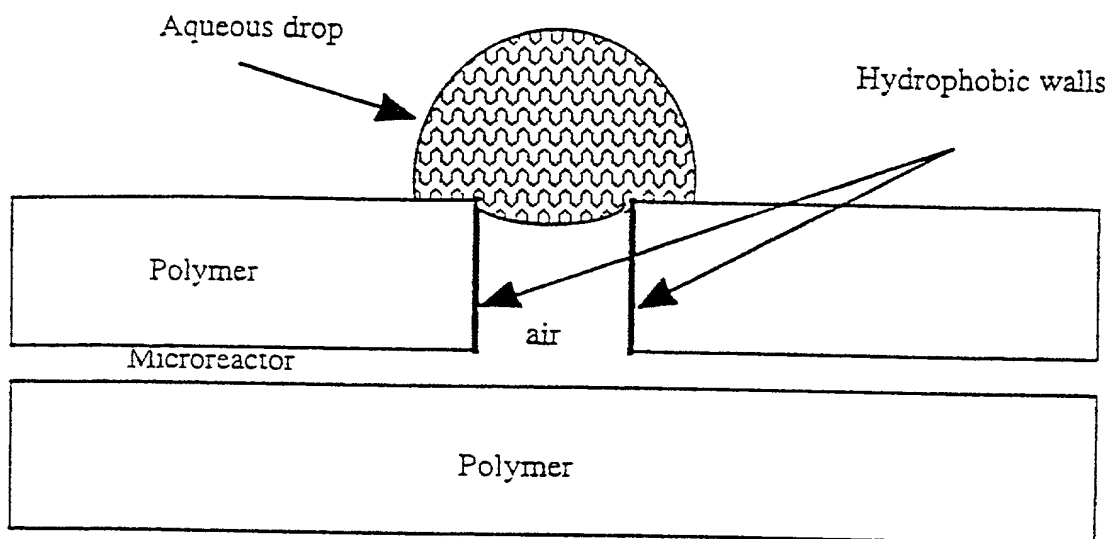
58. A method according to claim 57, wherein the sample  
is expelled from the reaction chamber by spinning the  
substrate at a greater angular velocity, in the range 10 to  
20 100,000 revolutions per minute, for a period of time in the  
range 1 to 100s.

59. A method according to any of claims 53 to 55, for  
operating an apparatus according to claim 7 or any claim  
appendant thereto, wherein the fluid entry force is provided  
25 by piston pressure.

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a)



b)

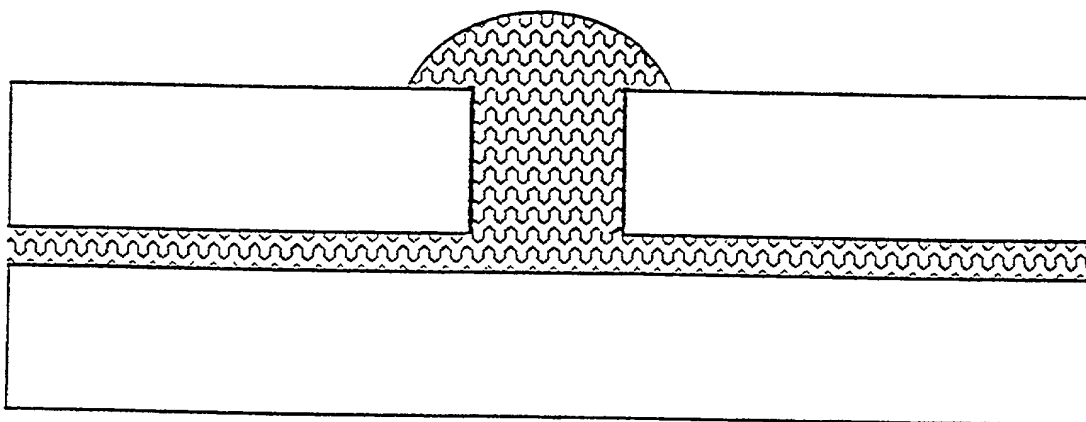
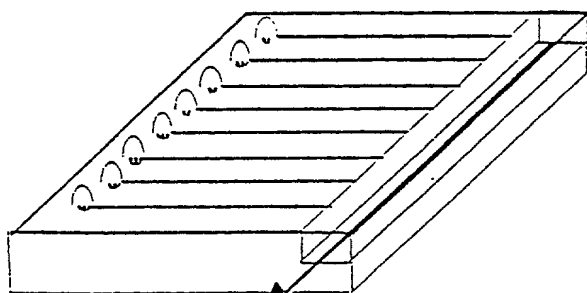
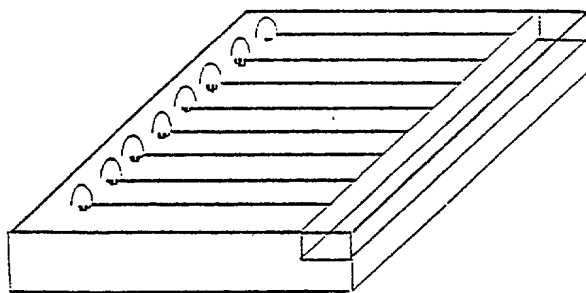
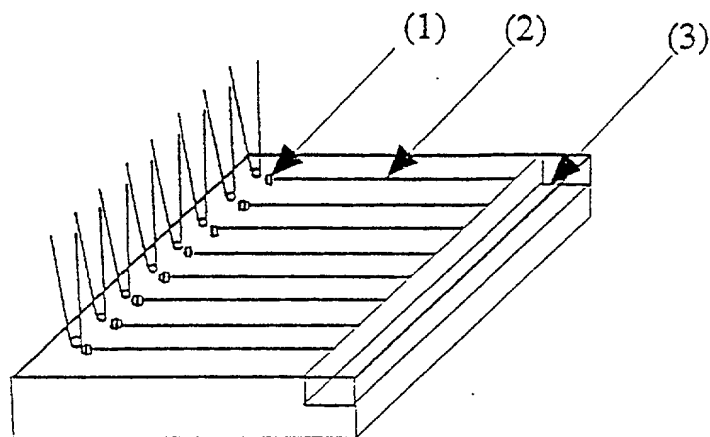
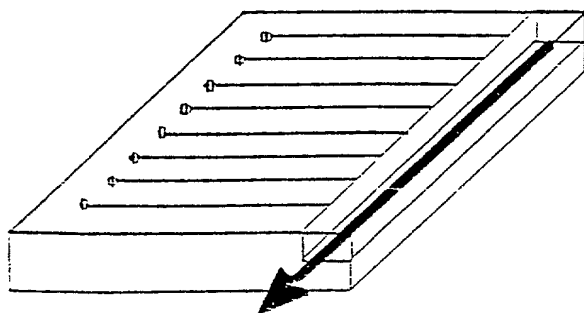


FIG 1

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Sample loading by slight aspiration



Washing by strong aspiration

FIG 2

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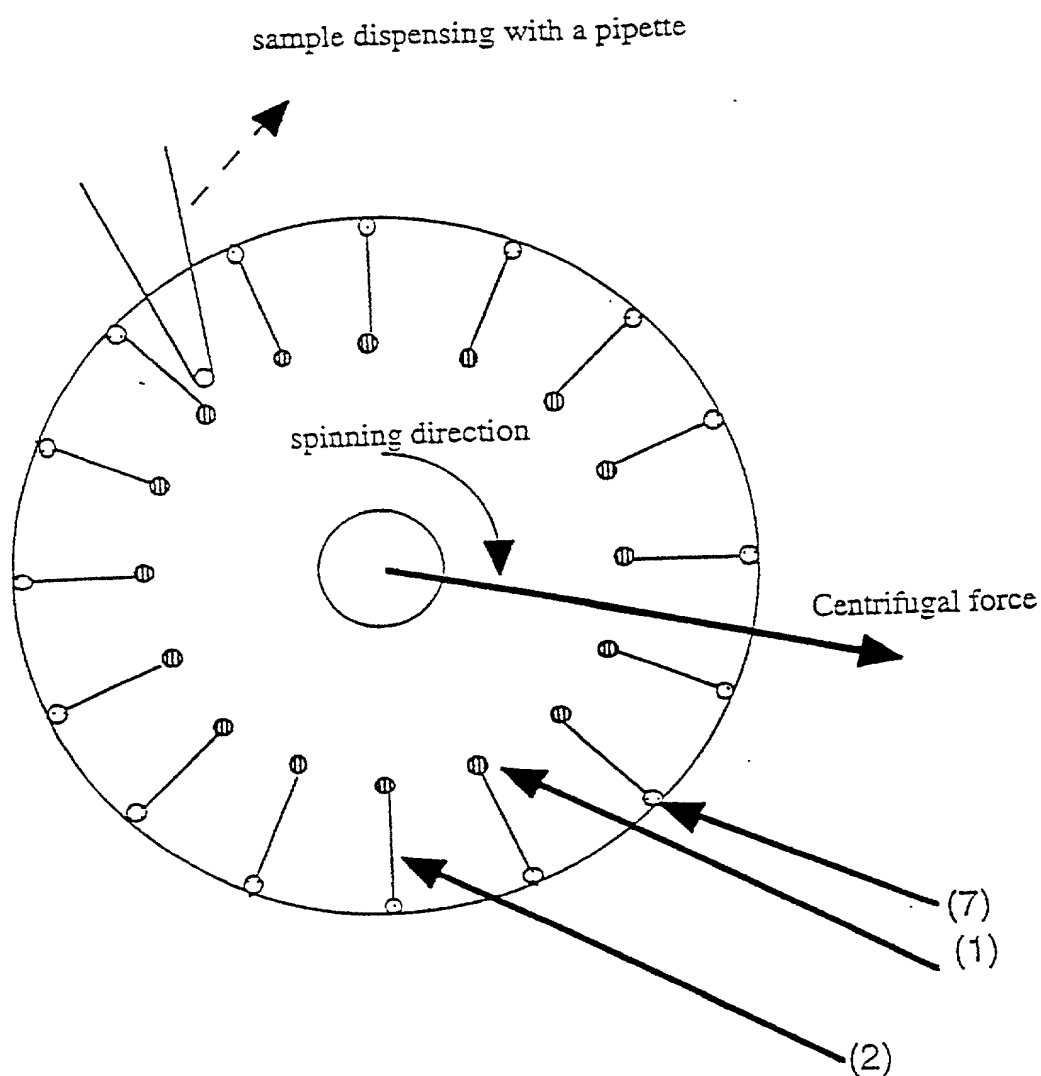


FIG 3

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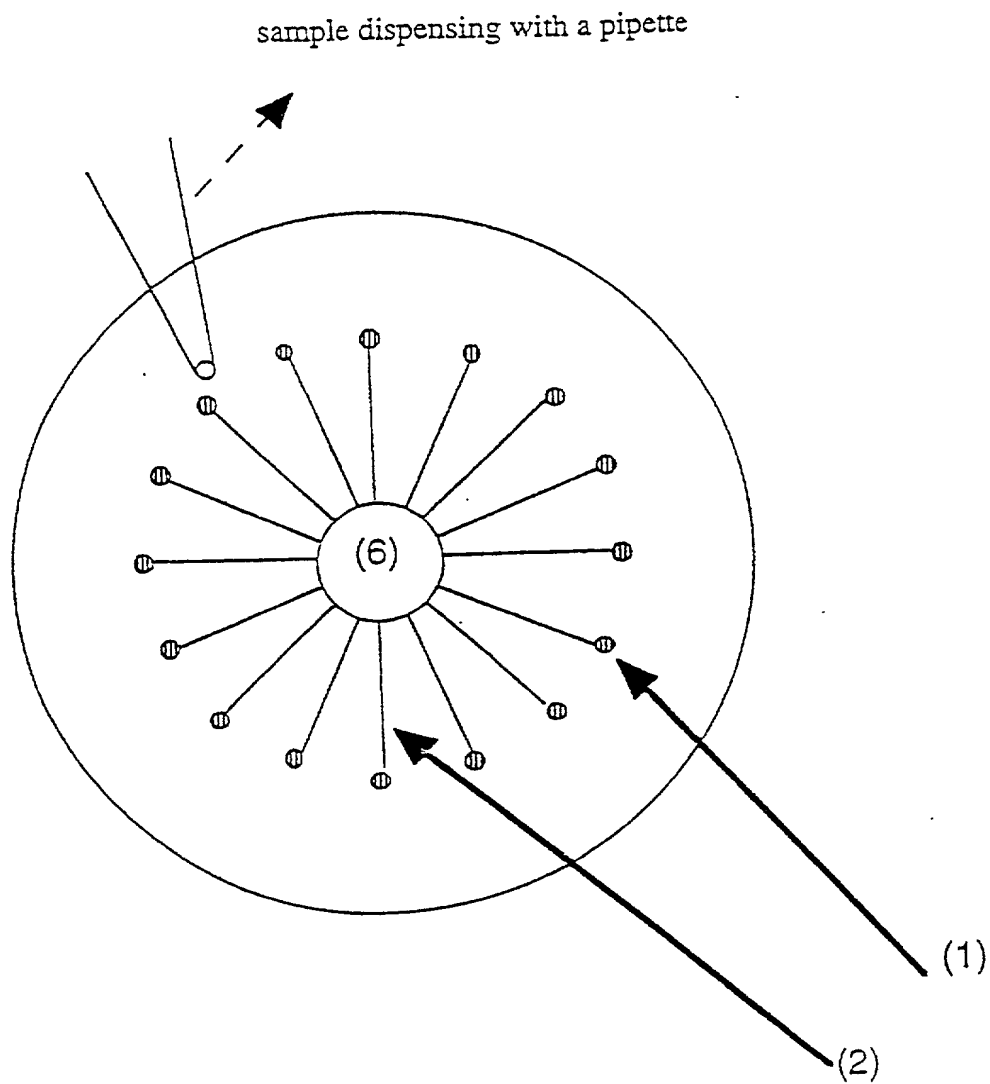


FIG 4

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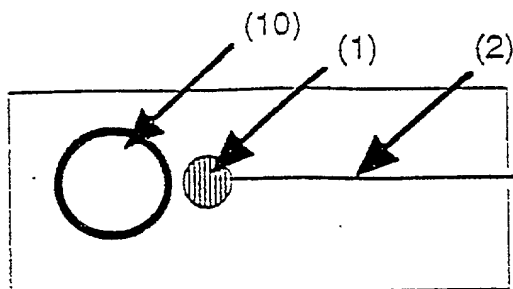


FIG 5a

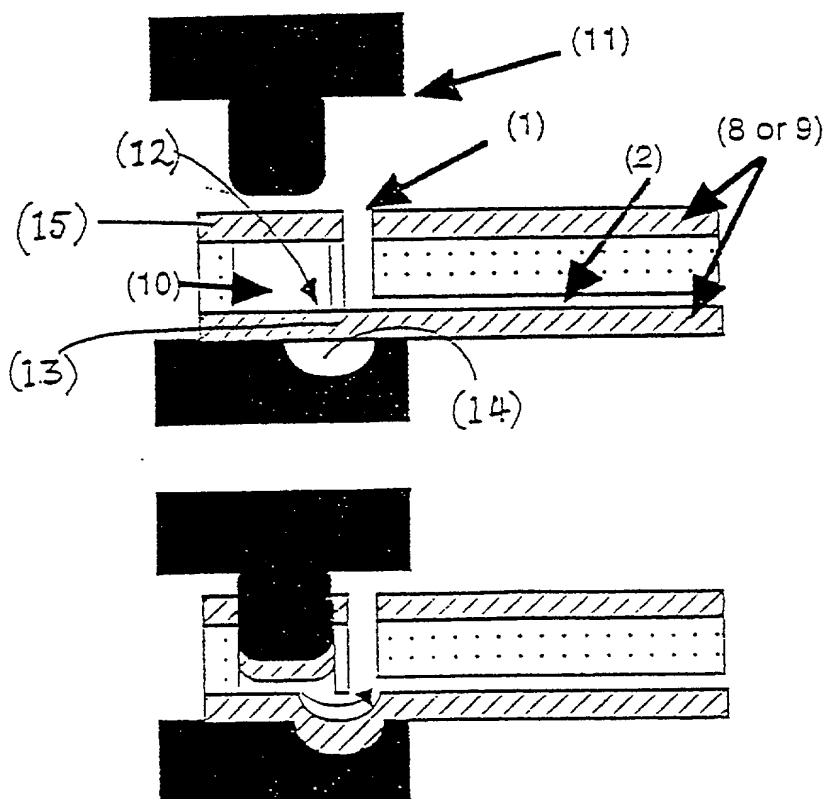
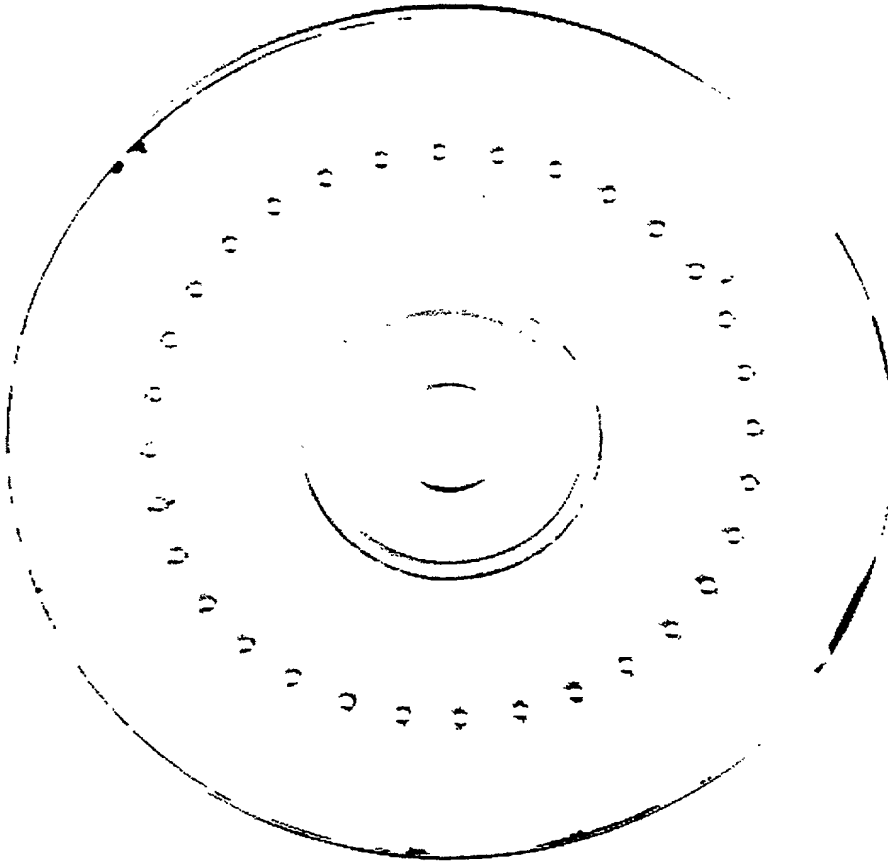


FIG 5b

Figure 6:



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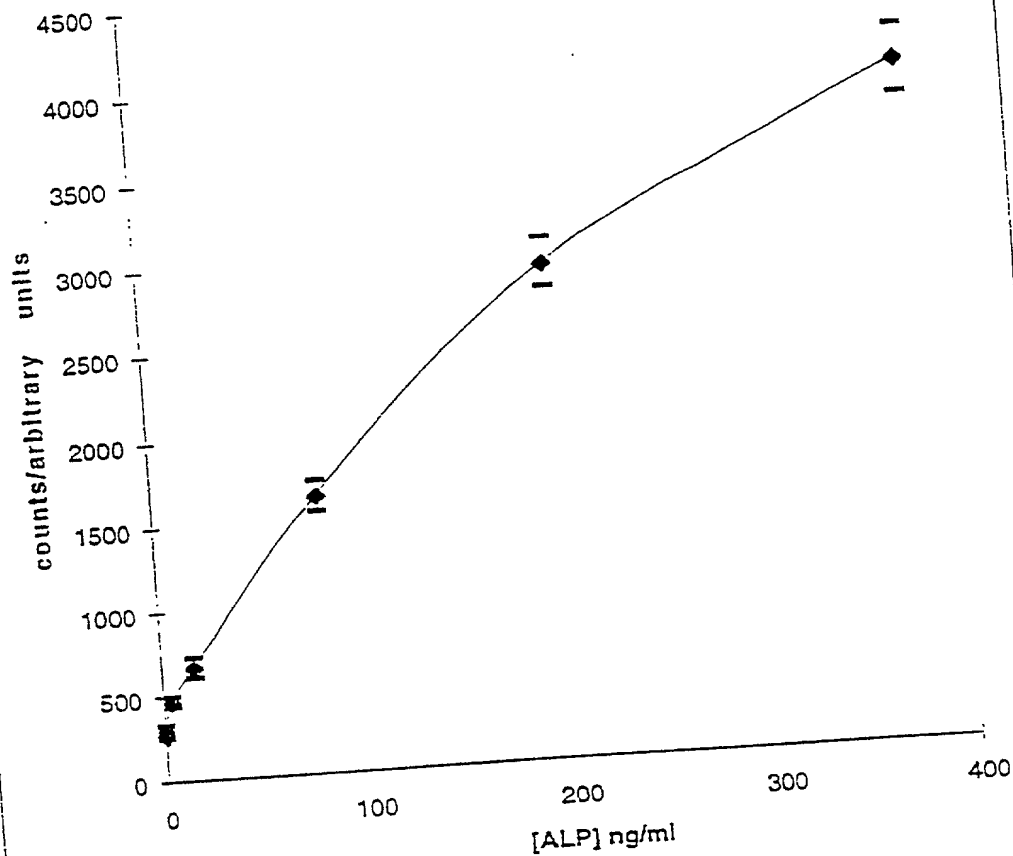


FIG 7

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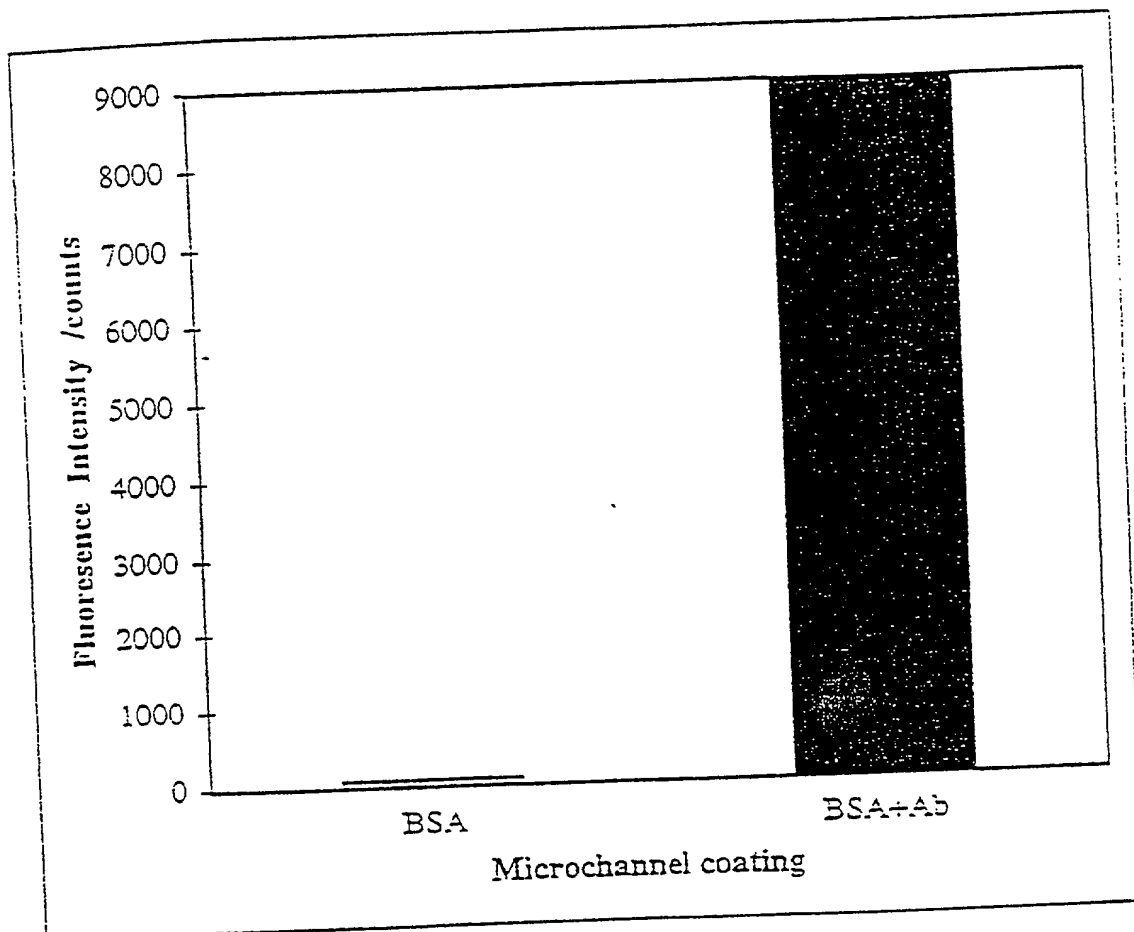
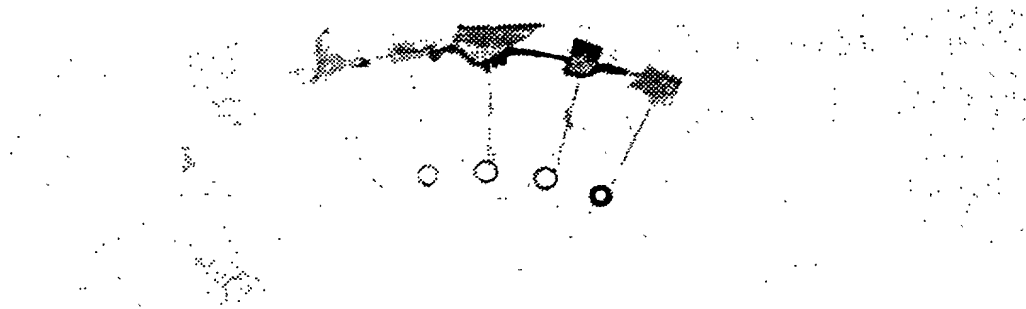


FIG 8

Figure 9:



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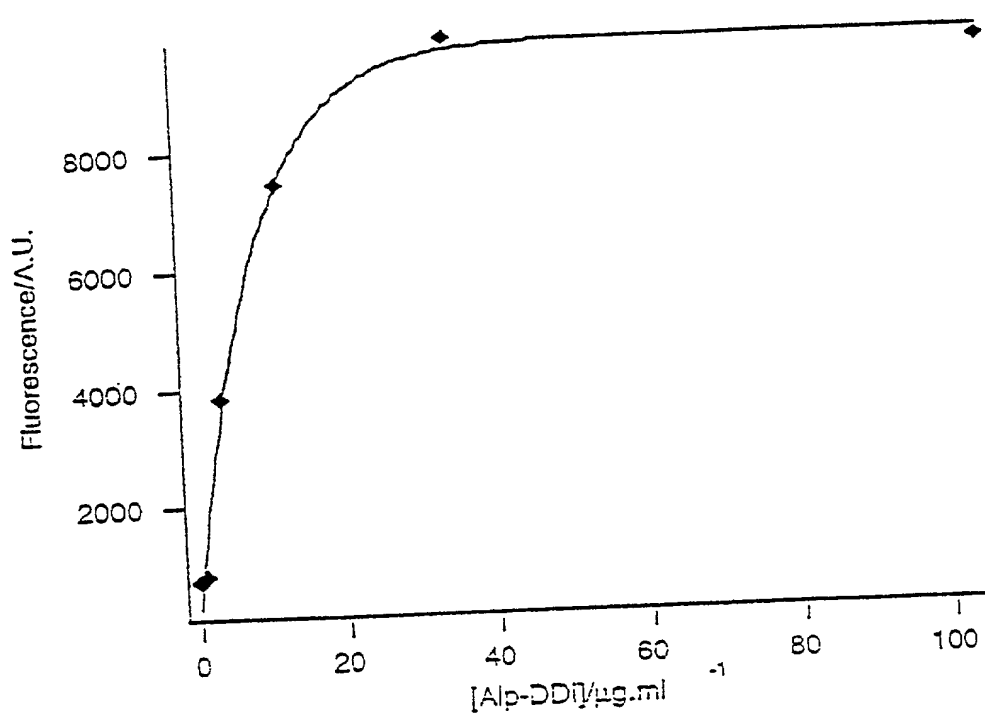


FIG 10

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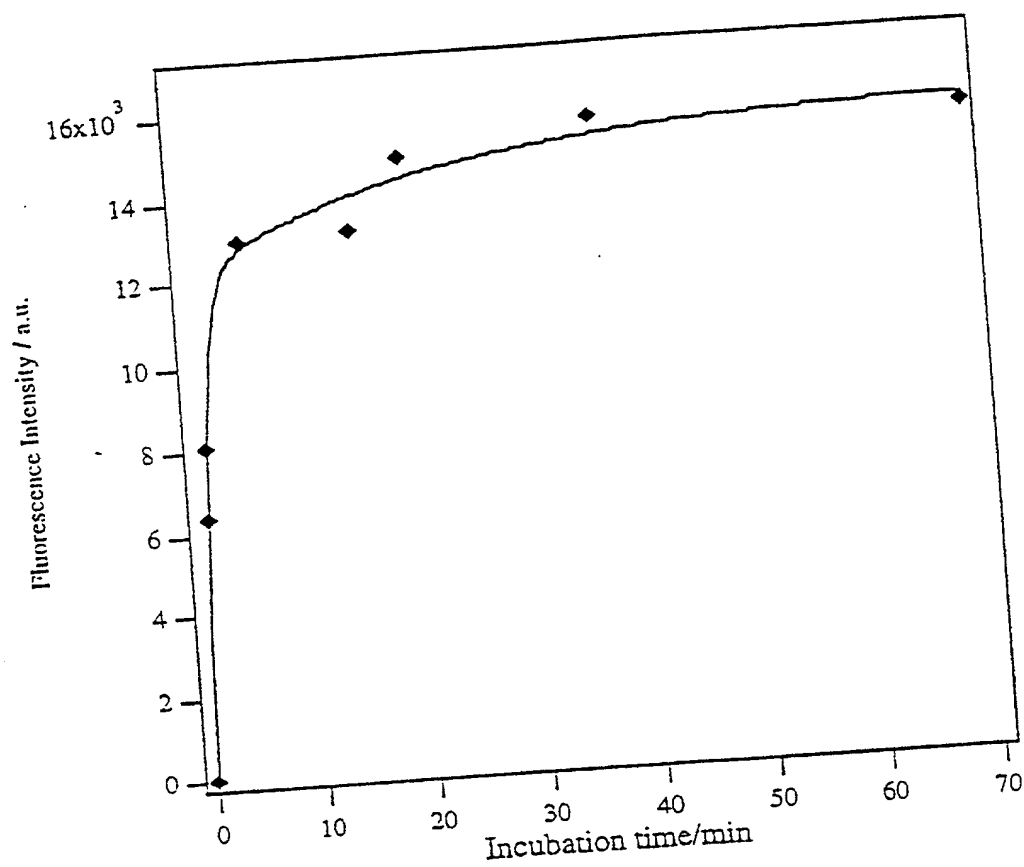


FIG 11

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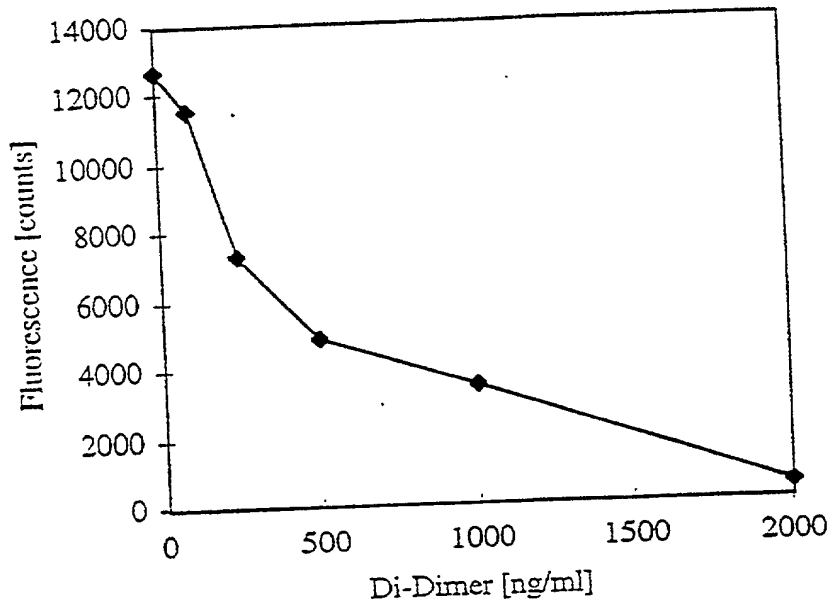


FIG 12

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**COMBINED DECLARATION AND POWER OF ATTORNEY**

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL,  
CONTINUATION, OR C-I-P)

---

As a below named inventor, I hereby declare that:

**TYPE OF DECLARATION**

This declaration is of the following type:

(check one applicable item below)

- ☐ original.  
☐ design.  
☐ supplemental.

**NOTE:** If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☒ national stage of PCT.

**NOTE:** If one of the following 3 items apply, then complete and also attach **ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.**

**NOTE:** See 37 C.F.R. § 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

- ☐ divisional.  
☐ continuation.

**NOTE:** Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. § 1.53(b) (application filing requirements — nonprovisional application).

- ☐ continuation-in-part (C-I-P).

**INVENTORSHIP IDENTIFICATION**

**WARNING:** If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

**TITLE OF INVENTION**

MICROSCALE TOTAL ANALYSIS SYSTEM

---

## SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b), or (c))

(a) ☐ is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) ☐ was filed on \_\_\_\_\_, as ☐ Serial No. 0 / \_\_\_\_\_  
or ☐ \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and application number (consisting of the series code and the serial number; e.g., 08/123,456);

"(2) name of inventor(s), serial number and filing date;

"(3) name of inventor(s) and attorney docket number which was on the specification as filed;

"(4) name of inventor(s), title which was on the specification as filed and filing date;

"(5) name of inventor(s), title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or

"(6) name of inventor(s), title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number; e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration."

Notice of July 13, 1995 (1177 O.G. 60), M.P.E.P. § 601.01(a), 6th ed., rev. 3.

(c) ☒ was described and claimed in PCT International Application No. PCT/EP00/02887, filed on March 28, 2000 and as amended under PCT Article 19 on \_\_\_\_\_ (if any).

**SUPPLEMENTAL DECLARATION (37 C.F.R. § 1.67(b))**

*(complete the following where a supplemental declaration is being submitted)*

- ☐ I hereby declare that the subject matter of the
- ☐ attached amendment
  - ☐ amendment filed on \_\_\_\_\_

was part of my/our invention and was invented before the filing date of the original application, above-identified, for such invention.

**ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

*(also check the following items, if desired)*

- ☒ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and
- ☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

**PRIORITY CLAIM (35 U.S.C. §§ 119(a)-(d))**

**NOTE:** *"The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by § 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. 119(b) must be filed in the case of an interference (§ 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in § 1.17(f). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. § 1.55(a).*

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

*(complete (d) or (e))*

- (d) ☐ no such applications have been filed.
- (e) ☒ such applications have been filed as follows.

**NOTE:** *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

(Declaration and Power of Attorney [1-1]—page 3 of 7)

09937690-030702

**PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION  
AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)**

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
GB	9907249.8 ✓	29 March 1999	<input checked="" type="checkbox"/> YES    NO <input type="checkbox"/>
			<input type="checkbox"/> YES    NO <input type="checkbox"/>
			<input type="checkbox"/> YES    NO <input type="checkbox"/>
			<input type="checkbox"/> YES    NO <input type="checkbox"/>
			<input type="checkbox"/> YES    NO <input type="checkbox"/>

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)  
(34 U.S.C. § 119(e))**

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER

FILING DATE

\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_  
\_\_\_\_\_/\_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S)  
UNDER 35 U.S.C. 120**

- ☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART (C-I-P) APPLICATION.

2020090652660

**ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION**

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

**POWER OF ATTORNEY**

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

Stanley B. Kita, Registration No. 24,561; George A. SMITH, Jr.,  
Registration No. 24,442; Wilson OBERDORFER, Registration  
No. 17,379; Mary E. BAK, Registration No. 31,215; Henry HANSEN,  
Registration No. 19,612; ~~and~~ Cathy Ann KODROFF, Registration No.  
(check the following item, if applicable) 33,980; and

William Bak, Registration No. 37,277.

- ☐ I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.
- ☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

DIRECT TELEPHONE CALLS TO:  
(Name and telephone number)

☐ Address

HOWSON AND HOWSON  
Spring House, Corporate Center,  
P.O. Box 457, Spring House,  
Pennsylvania 19477

(215) 540-9200

☐ Customer Number \_\_\_\_\_

## DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

Full name of sole or first inventor

1-00  
Hubert H. Girault  
(GIVEN NAME) (MIDDLE INITIAL OR NAME) FAMILY (OR LAST NAME)  
Inventor's signature Hubert Girault  
Date 31 October 2001 Country of Citizenship France ✓  
Residence Switzerland  
Post Office Address CH-1088 Ropraz, Switzerland CHX

Full name of second joint inventor, if any

2-00  
Frederic  Reymond  
(GIVEN NAME) (MIDDLE INITIAL OR NAME) FAMILY (OR LAST NAME)  
Inventor's signature Frederic  
Date 30th October 2001 Country of Citizenship Switzerland ✓  
Residence Switzerland  
Post Office Address Ch. des Marionnettes 15,  
CH-1093 La Conversion, Switzerland CHX.

Full name of third joint inventor, if any

3-00  
Joel S. Rossier  
(GIVEN NAME) (MIDDLE INITIAL OR NAME) FAMILY (OR LAST NAME)  
Inventor's signature Joel  
Date 30th October 2001 Country of Citizenship Switzerland ✓  
Residence Switzerland  
Post Office Address Ch. du Chamossaire 2,  
CH-1860 Aigle, Switzerland CHX.

(check proper box(es) for any of the following added page(s)  
that form a part of this declaration)

☐ **Signature** for fourth and subsequent joint inventors. *Number of pages added* \_\_\_\_\_

\* \* \*

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* \_\_\_\_\_

\* \* \*

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* \_\_\_\_\_

\* \* \*

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

\* \* \*

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added \_\_\_\_\_

\* \* \*

☒ Authorization of practitioner(s) to accept and follow instructions from representative.

\* \* \*

(if no further pages form a part of this Declaration,  
then end this Declaration with this page and check the following item)

☐ This declaration ends with this page.

09977690-030702

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR AUTHORIZATION OF ATTORNEY(S) TO ACCEPT AND FOLLOW  
INSTRUCTIONS FROM REPRESENTATIVE

The undersigned to this declaration and power of attorney hereby authorizes the U.S. attorney(s) named herein to accept and follow instructions from

JY & GW Johnson

*Name(s) of authorized representative(s)*

Kingsbourne House,

*Address*

229-231 High Holborn,

London WC1V 7DP ENGLAND

as to any actions to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney(s) and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorney(s) will be so notified by the undersigned.

(Added page to Combined Declaration and Power of Attorney for authorization of attorney(s) to accept and follow instructions from representative [1-24])

09937699 030702